EXPERIMENTAL STUDIES ON MITOCHONDRIA IN PLANT CELLS.

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INTRODUCTION.

During the last few years several investigations have been carried on with a view to utilizing the mitochondria as cytoplasmic indicators of cell activity. Reasoning on the basis of the fundamental differences which exist between the mitochondria and the nucleus it has been natural to assume that they may serve as clues to different types of activity. We are accordingly faced with the possibility of studying physiological and pathological processes from an entirely new angle, which may yield valuable and unexpected information. Thus far the results obtained from this new line of work have been contradictory and difficult to explain. On the one hand we have indications that, in certain instances, the mitochondria are very labile and respond easily to alterations in cellular activity (Busacca, '15; the Lewises, '15; Homans, '15; Scott, '16; and Goetsch ,'16); and, on the other, equally convincing observations to the effect that, in other cases, they are fixed and permanent cytoplasmic structures which do not evidence distinctive variations even under the influence of fairly advanced pathological conditions (Clark, '14; Strongman, '17; McCann, '18; and Rasmussen, '19, etc.). This condition has filled some investigators with enthusiasm and discouraged others. It should, however, only act as an incentive toward a systematic experimental study of mitochondrial variations.

Since the substantial similarity of mitochondria in plant and animal cells has already been demonstrated (N. H. Cowdry, '17) we have ample material to choose from. It is possible to apply certain experimental conditions to plants which are not feasible in the case of animals, and *vice versa*. For the purposes of this paper I have selected the sprouting rootlets of peas because the

normal properties of the mitochondria are well known and on account of the fact that the cells are relatively simple, owing to the absence of photosynthesis and extensive starch formation. In view of my failure to find any observations in the literature dealing with the experimental study of mitochondria in plant cells, I have planned my experiments so as to embrace a wide range of conditions in order to be able to define specific problems for further intensive study.

Material and Methods.

After the seeds had been sprouted and the plantlets experimented with, the radicles were treated by the Regaud formalin-bichromate method, and the sections (cut 4 μ) were stained with iron hematoxylin. It is of the utmost importance to use good and fresh formalin. In order to neutralize the free formic acid, which is so often present, I am in the habit of adding a small amount of magnesium carbonate to the stock solution. Attempts were made to control this method through the observation of living cells supra-vitally stained with janus green without very satisfactory results owing to the difficulty of getting the dye to penetrate the cells.

Since mitochondria exhibit considerable variations in different parts of the radicle I have confined my observations to the cortical cells between the elongating part and the root-cap. Even in this portion, however, neighboring cells occasionally exhibit, without apparent reason, variations in the size, shape and general appearance of the mitochondria, calculated to lead the unwary astray, and the cells near the surface react to experimental conditions and to fixation differently from those more deeply placed. It is important, also to bear in mind that mitochondria show a marked and constant regional variation in different parts of single cells so that serial sections are necessary for their study.

The usual appearance of the mitochondria in the cells under investigation is illustrated in Fig. 1. They will be seen to occur in the form of granules, short rods and filaments which show no indication of plast formation. They are distinctly more filamentous in the cytoplasm immediately beneath the cell membrane than in the region of the nucleus; this, however, is not so

well shown in the figure. The mitochondrial filaments near the nucleus show a tendency to become swollen and to stain more intensely with iron hematoxylin throughout their whole length or in certain restricted areas, as contrasted with those in the more peripheral parts of the cell.

The vacuoles occupy a considerable portion of the cytoplasm and sometimes contain a few mitochondria in their interior. These mitochondria, which are always darkly stained, enlarge into spherules of different sizes.

The effect of different experimental conditions was studied as follows:

Observations.

1. *Centrifuging.*

Plantlets were centrifuged for one hour with results as indicated in Fig. 2. The nucleus appears to have been thrown against the cell wall and is in some cases quite flattened. While a thin layer of protoplasm remains everywhere in contact with the cell wall, the greater portion of it has assumed a postion between the nucleus and the vacuole. It is of shreddy consistency and contains enlarged mitochondria.

I have seen no indications of the existence of a difference in specific gravity between the mitochondria and the protoplasmic ground substance in the cortical cells of the pea radicle as described by Fauré-Fremiet ('13, p. 602) in *Ascaris*. Key,[1] using also the centrifuge method, failed to detect any difference in the specific gravity of the mitochondria and the protoplasmic ground substance of nerve cells. On the other hand Beckwith ('14, p. 216) succeeded in separating the cytoplasmic constituents in the eggs of *Hydractinia* into three layers: first, the oil cap, second, a layer of clear protoplasm and, lastly, a mingled mass of yolk and mitochondria. These observations indicate, probably, the existence of variations in the fluidity of the ground substance, not of differences in the mitochondria themselves.

2. *Plasmolyzing Agents.*

Normal plantlets of two days growth were placed so that their radicles were immersed in a 20 per cent. solution of cane sugar

[1] Quoted from E. V. Cowdry ('18, p. 84).

for 48 hours. The resulting plasmolysis is illustrated in Fig. 3. It will be noted that the mitochondrial filaments have almost completely disappeared leaving only a comparatively few enlarged spheres, sometimes with faintly staining centers. The vacuoles show no mitochondrial content. None of the control peas subjected to the same treatment survived and the assumption is, in agreement with Guilliermond ('18), that the mitochondria enlarged after the death of the cell.

3. *Desiccation.*

Normal plantlets were deprived of water at room temperature for 36 hours, which resulted in considerable shrinkage of the growing root-tips. On examination the cells were found to contain for the most part granular, and rather swollen mitochondria (Fig. 4). The vacuolar membrane seems to have disappeared and the protoplasm exhibits great variation in intensity of staining reaction, as do also the contained mitochondria, which in other respects appear to be quite normal.

4. *Illumination.*

Some plantlets were kept in complete darkness, while others of the same age were subjected daily to strong sunlight without affecting in any distinctive way the mitochondrial content of their radicles. It would seem, therefore, that the mitochondria in the cortical cells of the pea radicle are not directly concerned with chemical processes which are accelerated and retarded by variations in illumination. Unfortunately the chlorophyll-containing plumules were not examined.

5. *Increased Temperature.*

Plantlets were heated in a thermostat at various temperatures, care being taken to avoid evaporation.

The first changes were observed in radicles exposed to a temperature of from 43 to 46 degrees C. for 3 hours (Fig. 5). All the filamentous mitochondria disappear and their place is taken by numbers of small faintly staining granules, though a few large and deeply staining spherules may still be seen. It is possible that this segmentation of the mitochondria might not have

occurred had the temperature been applied gradually instead of suddenly.

When heated to a temperature of from 47 to 49 degrees C. for 30 minutes the above mentioned granules show a darker stain and appear fewer in number (Fig. 6). Many peculiar, irregular, darkly staining masses may be seen within the nuclei which are difficult to interpret. Controls showed that the tips were all killed, but the remaining portions of the plantlets recovered in a few cases.

When exposed to a temperature of from 65–73 degrees C. for 40 minutes the intensely staining granules become greatly reduced in number (Fig. 7), the cytoplasm varies greatly in consistency and staining reaction, and the nuclei lose all traces of deeply staining nucleoli and granules. None of the controls of the experiment survived.

In my experience, therefore, plant mitochondria are not rendered vesicular through increase in temperature in the same way as the mitochondria in the tissue cultures experimented with by the Lewises ('15). I have also been quite unable to confirm Policard's ('12, p. 229) interesting observation that in some glandular cells the mitochondria become partly dissolved, when heated to a certain temperature, leaving an unstained residue behind which he believes to represent their albuminous component. According to Jost ('07, p. 140), however, albumins proper occur only occasionally in plant cells.

6. *Decreased Temperature.*

Plantlets were exposed to a temperature of 11 degrees C. in an ordinary ice box for 4 days and preparations were made of their radicles. The cells showed very little change (Fig. 8); but it was possible to detect a slight tendency toward clustering of lightly stained filamentous mitochondria in the perinuclear area, and further, that these filaments do not enlarge and stain more intensely than those in other parts of the cytoplasm as they do in normal cells. The experiments which I have already mentioned on the effect of illumination preclude the possibility of attributing this change in the mitochondria to the darkness of the ice box.

An exposure to the same temperature for eighteen days apparently brings about a breaking up of the mitochondrial filaments into short rods and granules which stain intensely (Fig. 9). The protoplasm is also seen to be distinctly reticulate, the mitochondria occurring in the denser strands. The more intense staining of the nucleolus, shown in the figure, appears to be an individual variation not associated with exposure to cold.

Other plantlets which were placed in a freezing mixture of ice and salt in the ice box for 20 hours show more advanced alterations (Fig. 10), the mitochondria remaining about the same and the protoplasm showing many scattered vacuoles, the reticulate appearance having disappeared.

The presence of distinctly filamentous mitochondria after a sojourn of 4 days in the ice box tends to support Dubreuil's view ('13, p. 137) that filamentous mitochondria are indicative of rest and granular ones of active multiplication by division, because it is safe to assume that the chemical changes in which the mitochondria are concerned share in the general retardation occasioned by reduced temperature. Other important considerations however show that this generalization does not hold (E. V. Cowdry, '18, p. 67).

7. *Submergence in Water.*

Entire plantlets were submerged in ordinary tap water for 24 hours and thus deprived of the regular amount of oxygen. The chief alterations are manifest in the inner cells of the cortex (Fig. 11), in which the mitochondria have broken up into rather large short rods and granules which vary in their staining reaction. The more superficial cells of the cortex, on the other hand, show no characteristic changes, the mitochondria retaining their normal filamentous shape. The controls showed a varying degree of vitality, about 50 per cent. being killed.

8. *Restricted Air Space.*

Plantlets were placed in a bottle, containing an air space of about 15 c.c. and tightly closed with a paraffin-coated cork. Preparations were made after 24 hours which showed a reversal of the changes due to submergence; for in this case the altera-

tions were more marked in the outer cortical cells, which present complete chondriolysis, than in the deeper ones. In the middle cells of the cortex the mitochondria exhibit great polymorphism as illustrated in Fig. 12, rings, spheres, rods, granules, and dumb-bell shaped forms being visible. Variations in staining reaction are of common occurrence and the mitochondria have lost their tendency to cluster about the nucleus. The inner cells, on the contrary, contain the usual thin filaments with some plast-like forms (Fig. 13).

When the same treatment is continued for two days the alterations become still more pronounced. The cells of the plerome are illustrated in Fig. 14. The mitochondria are very scanty, the protoplasm shows signs of disorganization and the nuclei are loaded down with a granular deposit. The controls from this series all died so that we are unquestionably dealing with death changes.

These observations seem to be in accord with the view first advanced by Kingsbury ('12, p. 46) and supported by Mayer, Rathery and Schaeffer ('14, p. 619) that the mitochondria play an active part in protoplasmic respiration.

9. *Chloroform.*

Plantlets were exposed to the vapor of chloroform, in a covered Petri-dish of 100 c.c. capacity for 45 seconds without the mitochondria undergoing any marked alterations. Continuing the same treatment for 2½ minutes, however, brings about distinct changes (Fig. 15), only a thin layer of protoplasm remaining intact, in which may be distinguished a few scattered mitochondria with very vague outlines. The vacuole is filled with an intensely stained granular deposit without any trace of mitochondria. Plasmolysis is fairly advanced. This treatment resulted in the death of the root tips of the control plantlets. It is interesting to compare this condition to the well known immunity of mitochondria to the action of chloroform after fixation.

10. *Ether.*

On exposure to the vapor of ether, under the same conditions, for 3½ minutes no distinctive changes were observed in the

mitochondria. After exposure for seven minutes the mitochondria in the cortical cells are seen to be shrunken and of irregular outline, and to be darkly stained (Fig. 16). Peculiar, irregular, darkly staining masses also appear in the nuclei which are difficult to interpret. The protoplasm has changed in consistency in the cells, but in those of the early meristem and root-cap it is shrunken and stained uniformly and intensely in these preparations, so that the internal structure is difficult to make out. This is not due to incomplete differentiation. The cell walls in these regions seem to have undergone partial fragmentation.

Controls subjected to the same ether treatment and afterwards placed on damp absorbent cotton grew vigorously with the plumule much greater than in the case of other peas sprouted at the same time which had not been treated with ether. According to Jost ('07, p. 195) weak etherization accelerates respiration while strong etherization inhibits it, by killing the cells. It will be seen that growing the plantlets in lecithin has the reverse action in stopping the growth of the plumule abruptly and in inhibiting chlorophyll production.

The mitochondria are if anything less affected by 7 minutes in ether vapor than by 45 seconds exposure to vapor of chloroform.

11. *Glycerin.*

Plantlets were, as usual, sprouted normally and then placed in a 10 per cent. solution of glycerin in tap water for 18 hours. This treatment brings about a complete chondriolysis of all the mitochondria in the root tip (Fig. 17) and may be compared with the effect of extreme heat (Fig. 7) and of restricted air space for two days (Fig. 14), though the condition of the ground substance is somewhat different in these conditions. None of the controls survived.

12. *Lecithin.*

Plantlets were placed so that their radicles were in intimate contact with sphagnum moss, thoroughly soaked with a 1 per cent aqueous solution of lecithin, for 24 hours. (The lecithin used was a pure variety obtained from Dr. Levene of the Rockefeller Institute.) The cells in these preparations present a re-

markable picture (Fig. 18). They are loaded with a multitude of granules and filaments, some of which appear to be swollen and elongated to a relatively large extent, indicating perhaps imbibition of lecithin from the surrounding fluid and subsequent incorporation in the mitochondrial substance. It is to be further noted that these enlarged mitochondria are never truly vesicular; their staining reaction, in some cases, appears to be fainter than that of the definitive mitochondria, which presumably have not approached the nucleus and which are changed to a comparatively minor degree.

Other plantlets grown on blotting paper saturated with lecithin solution exhibited the same appearance.

Radicles, of control plantlets subjected to the same treatment, grew vigorously, but the growth of the plumule was abruptly stopped and chlorophyll formation ceased entirely. Compare this with the growth and increased chlorophyll production under the influence of ether.

This association of lecithin with the enlargement of mitochondria is of interest from several points of view. In the first place, Russo ('12, p. 215) claims to have been able to increase the mitochondria in the oöcytes of the fowl through the injection of lecithin. Unfortunately, however, the technique which he employed is open to criticism on account of the lack of specificity of his staining reactions. In any case, the parallelism between his observations and my own is interesting and may be significant. Moreover, Löwschin ('13, p. 203; '14, p. 269) is credited with the making of artificial mitochondria with lecithin in different salt and albumin solutions, which in some respects resemble true mitochondria very closely. Many other observations might be cited which indicate that mitochondria are allied in composition to substances of the phosphatid group.

Discussion and Conclusion.

In considering the effect of various experiments on mitochondria we must bear in mind the normal variation. They vary in diameter, length and appearance in the same cell and in different parts of the same root-tip where the cells of the plerome, periblem, root-cap, epidermis and meristem each have some peculiar

mitochondrial appearance. The elongated cells of the periblem are marked by mitochondria differing from those of the younger cells of the same portion. It is impossible to find two rootlets, although growing under precisely similar conditions, in which there is not some difference in the mitochondrial contents of cells from similar portions.

We find also that there is much variation in the manner in which mitochondria react to experimental conditions in different parts of the same rootlet and also in rootlets of different stages of growth. The thin, lightly stained filaments, rods and granules are changed by many causes, the granules of older cells are more resistant, and spheres sometimes remain in the protoplasm when all traces of mitochondria have disappeared.

Segmentation is peculiar to mature filaments and occurs from many causes. It is even an individual variation in plantlets grown under precisely similar conditions. I have seen no indication that the resulting granules elongate to form new filaments or that mitochondria increase through elongation of original granules and the segmentation of the resulting filament.

Mitochondria normally agglutinate and form lipoidal masses in close proximity to the nucleus. These masses seem to disappear and to go into solution in older cells which contain only the persistent granular mitochondria. Scott ('16, p. 249) has also observed agglutination of mitochondria in the cells of the pancreas of animals poisoned by phosphorus.

Mention should here be made of the spherical inclusions so commonly seen in the vacuoles of early cortical cells, the origin of which is, in all probability, mitochondrial. They resemble very closely the spheres in cells at the base of the rootlet which clearly arise from the dissolution of aleurone grains. They both disappear in a similar manner in the median portion of the rootlet, at a distance from their origin. They probably are very similar in composition, for aleurone grains contain all the elements supposed to exist in mitochondria and probably are the source of the continuous supply of the mitochondrial matter supplied by the cotyledons.

The composition and consistency of the protoplasm has some effect on the behavior and appearance of mitochondria in normal

as well as under experimental conditions. MacDougal (p. 199) in referring to the living matter of plant cells, says: "Living matter is composed mainly of pentosans and albumins, or albumin derivatives with lipins as a minor component. The proportion of the main components may vary from nearly one hundred per cent, to nearly zero." In reticulate or alveolate protoplasm, mitochondrial granules are found in the strands of the network or in the more condensed portion of the protoplasm and never in the meshes or alveoli. Filaments, on the other hand, are seen only where the "main components" are intimately mixed and the protoplasm appears to be homogeneous. The differences in chemical constitution, structure of the cell wall and in other particulars, make it difficult to compare the reactions of plant and animal cells to experimentation.

In reviewing the several experiments it will be seen that mitochondria are changed to an abnormal degree only under severe conditions which either kill the cell or render its recovery very improbable. Even when the cell has been killed its general appearance varies in almost every case, the mitochondria however are very much reduced in number or disappear entirely by apparent solution, or in the case of plasmolysis through the prior appearance of vesicles.

In the experiments with submergence and restricted air space, where a sufficient supply of oxygen for respiration has not been supplied, the mitochondria are changed in a somewhat similar manner but the location of the changes is reversed. Exposure to the vapors of ether and chloroform produce very different results, and even in cells stimulated by ether vapor or by lecithin, mitochondria are very different in appearance, though increased growth results in both cases.

I hope in my next paper to make an intensive study of the influence of lecithin upon the mitochondria in plant cells.

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EXPLANATION OF PLATES.

All the figures have been drawn with the aid of a Zeiss apochromatic 1.5 mm. objective, a Spencer ocular No. 10 and a camera lucida.

Cortical cells, between the root-cap and the elongating portion of the sprouting pea radicle, were selected. The radicles were fixed by the Regaud formalin-bichromate method and the sections stained with iron hematoxylin.

PLATE I.

FIG. 1. Normal cells of the cortex near the meristem of a radicle about 10 mm. long. The mitochondria are filamentous and granular, and exhibit a varying intensity of staining reaction. They show little tendency to cluster around the nucleus or to enlarge. The vacuoles contain mitochondrial granules.

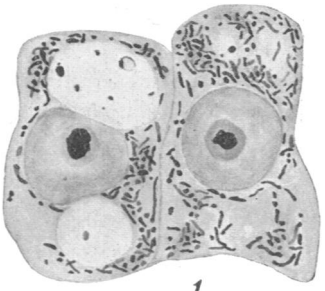
FIG. 2. Showing the effect of centrifuging for one hour. The radicle is about 6 mm. long and has not yet elongated. Mitochondria are few and unaffected in position in the protoplasm and in the vacuoles they are evenly distributed among protoplasmic shreds. The heavy nucleus adjoins the cell wall while the greater part of the protoplasm lies between the nucleus and the vacuole. The continuity of the protoplasmic lining of the cell wall is unbroken.

FIG. 3. Plasmolysis caused by immersion of the radicle, about 7 mm. long, in a 20 per cent. solution of cane sugar, for two days. Mitochondria are few in number and have enlarged into spherules with centers lightly stained. The protoplasm is very unevenly stained and contains no vacuoles. Intensely stained masses appear at the periphery of the nucleus.

FIG. 4. Illustrating the effect of drying the plantlet for 36 hours. Cell from a radicle about 9 mm. long. The mitochondria, differing in size and intensity of coloration, are granular and show no inclination to approach the nucleus. The nucleolus is only faintly stained. Other cells show a large vacuole with globules of protoplasm and many mitochondrial spheres.

FIG. 5. The plantlet was heated to a temperature of from 43 to 46 degrees C. for three hours. The radicle is short, being only 5 mm. long. Mitochondrial granules are very numerous and lightly stained. The plast-like forms are characteristic of the Alaska, a green variety of pea. The protoplasm is uniform but, where thin, it becomes alveolate.

FIG. 6. Plantlet exposed to a temperature of from 47 to 49 degrees C. for 30 minutes. Cells from a radicle 9 mm. long. Mitochondria are few and granular with increased intensity to stain. The strongly stained masses at the periphery of the nucleus probably result from the increased temperature.



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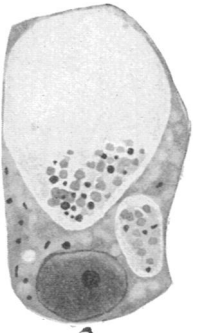

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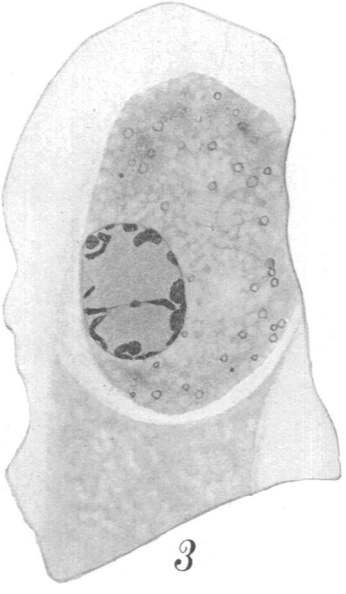

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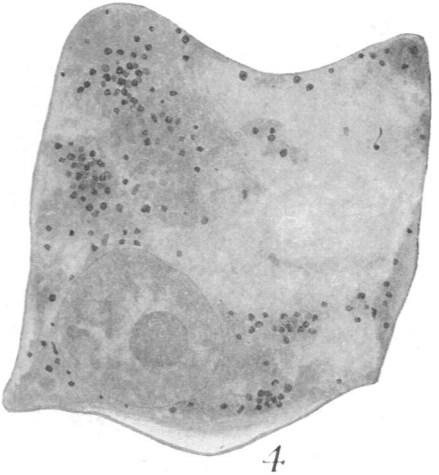

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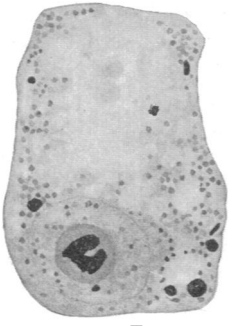

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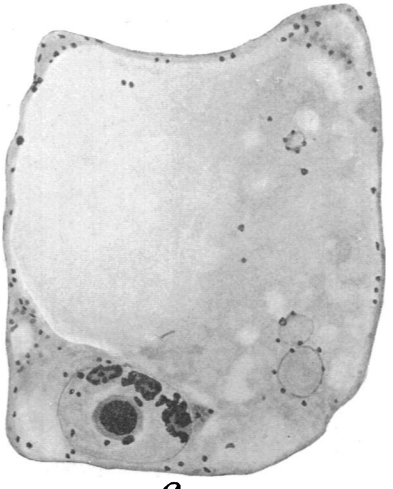

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PLATE II.

FIG. 7. A plantlet of the marrowfat variety was heated to a temperature of from 65 to 73 degrees C. for 40 minutes. A cell from a radicle 10 mm. long is figured. A few mitochondrial granules still persist with others not so strongly stained and almost indistinguishable in the granular or mealy protoplasm. The black granules at the periphery of the nucleus have lost their stain.

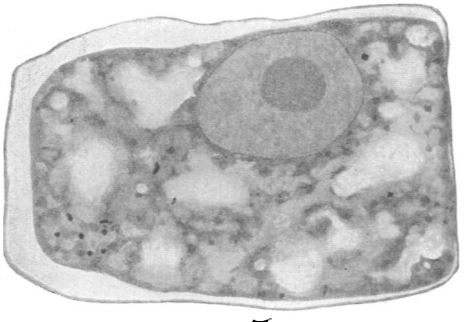
FIG. 8. The plantlet was exposed to a temperature of about 10 degrees C., in an ice box, for 4 days. The cell figured is from a radicle 9 mm. long and shows filaments, not strongly stained, clustering about the nucleus and remaining unchanged, although some show strongly stained nodes. The vacuoles in other cells contain intensely stained and enlarging mitochondrial spheres and shreds of protoplasm.

FIG. 9. Showing effect of prolonged exposure to a temperature of about 10 degrees C., in an ice box, for 18 days. The cell figured is from a radicle 6 mm. long. The filaments have, in most cases, segmented into intensely stained granules and short rods. Those in the vicinity of the nucleus are enlarged. The protoplasm is distinctly reticulate.

FIG. 10. Exposed to the low temperature of a freezing mixture of ice and salt and gradually restored to room temperature. The cell from a radicle 5 mm. long, shows rather minute granular mitochondria of varying intensity of stain. The protoplasm is distinctly alveolate. No mitochondrial granules occur in the alveoli.

FIG. 11. The plantlet was submerged in water for 24 hours. In the cell figured from the inner cortex of a radicle 9 mm. long, are many enlarged granules and a few short rods varying in intensity of stain and evenly distributed in the protoplasm. There are no filaments in this cell, but in cells of the outer cortex are many lightly stained filaments which are segmenting into shorter rods. The vacuoles contain many intensely stained mitochondrial spheres.

FIG. 12. The plantlet was enclosed in an air-tight space of about 15 c.c. for one day. A cell from the middle cortical layers of a radicle 5 mm. long was selected. The mitochondrial filaments have assumed the form of short rods, spheres and rings which show no tendency to approach the nucleus. They lose their stain entirely in the outermost cells. The vacuoles are large and contain few and sometimes no enlarged mitochondria.



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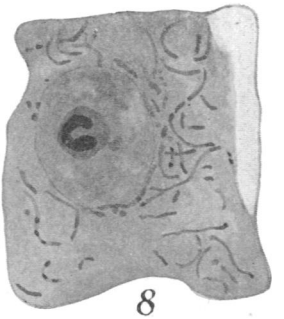

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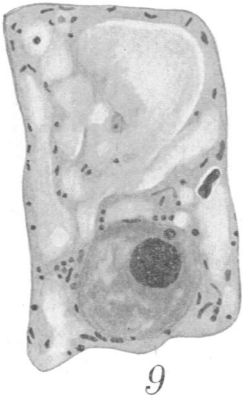

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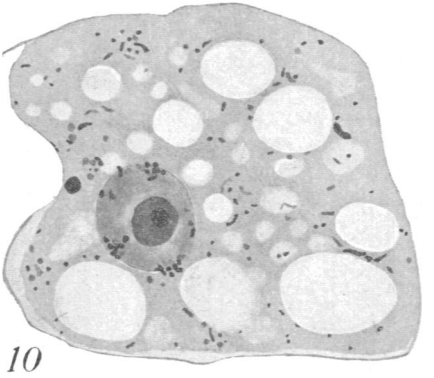

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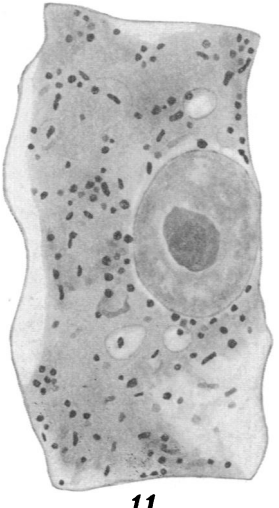

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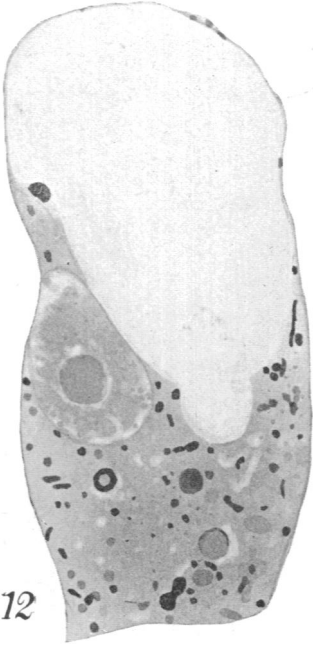


12

N. H. COWDRY

PLATE III.

FIG. 13. The cell figured is from the inner cortex of the same section as Fig. 12. Mitochondria here are almost normal. Filaments are seen with no tendency to approach the nucleus and contrasting strongly with their altered appearance in the middle and other layers.

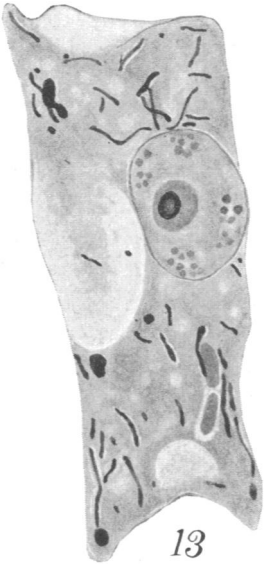
FIG. 14. Plerome cell from a radicle 6 mm. long and exposed to the same confined air-space for two days. Mitochondria have, in many cells, completely disappeared. There are, however, in the cells of the plerome a few lightly stained rods and granules, with some intensely stained, enlarged plast-like bodies. The nucleus shows many strongly stained granules at its periphery.

FIG. 15. Cell from the cortex of a plantlet exposed to the vapor of chloroform, for 2½ minutes in a Petri-dish of 100 c.c. capacity. The radicle is about 7 mm. long. Mitochondria have disappeared leaving no trace except a few lightly stained spherules and dark blotches in the protoplasm. The vacuole is filled with a comparatively darkly stained coagulum.

FIG. 16. Cell from a radicle 10 mm. long exposed to the vapor of ether for 7 minutes under the same conditions as Fig. 15. Mitochondrial granules and rods are very distinct but are irregular in outline. The protoplasm has greatly changed in consistency and appearance.

FIG. 17. The plantlet, grown normally, was placed so that its radicle was immersed in a 10 per cent. solution of glycerine for 18 hours. A radicle 7 mm. long was selected. The cells show a complete disappearance of mitochondria leaving no trace except indistinct darker staining areas in the protoplasm.

FIG. 18. Plantlets grown normally were placed so that their radicles were in contact with a one per cent. solution of lecithin for one day. A radicle 25 mm. long was selected. Mitochondria exhibit a very marked increase in number and size.



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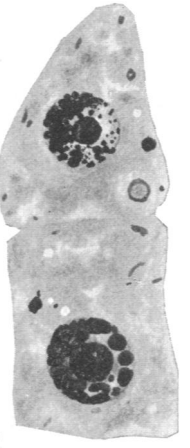

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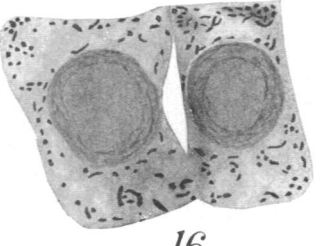

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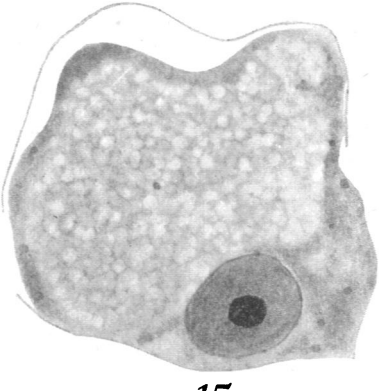

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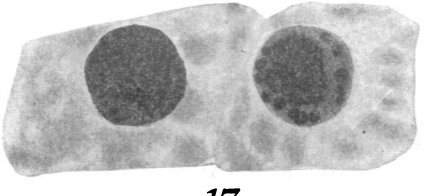

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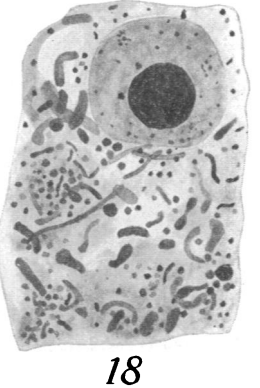

18